Brody, David and Thomas Esparza 2021

Dr. David Brody and Mr. Thomas Esparza Oral History

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NIH News Release, December 22, 2020: NIH neuroscientists isolate promising mini antibodies against COVID-19 from a llama

Dr. David Brody and Mr. Thomas Esparza

Behind the Mask

January 14, 2021

Barr: Good morning. Today is January 14th, 2021 and I have the pleasure of speaking to Dr. David Brody. Dr. Brody is the Director of the Center for Neuroscience and Regenerative Medicine at the Uniformed Services University of the Health Sciences and Mr. Thomas Esparza. Mr. Esparza works at the Henry M. Jackson Foundation for the Advancement of Military Medicine. Both Dr. Brody and Mr. Esparza have strong affiliations with the National Institute of Neurological Disorders and Stroke. Thank you very much for being with me today and talking about your COVID research.

Brody: Thank you for the opportunity.

Barr: To begin, can you please define what nanobodies are and why they are so important to discuss in the context of COVID?

Brody: Sure, nanobodies are very small fragments of an antibody that comes from an animal like a llama, or a camel, or a dromedary, or other animals in that family. The reason they are called nanobodies is because nano is a word that usually means really small and these are really small. They are about one-tenth of the size of normal antibodies and the reason we think they are important and the reason we are interested in them is, in fact, because they are so small. They are much easier to make because they are small and you can, in principle, inhale them. You can spray them and inhale them, and they will work, whereas with regular antibodies it doesn't really work very well.

Our main interest in the COVID-19 domain has been in making nanobodies that will block the virus, and, in the future, we hope, they will be useful as a spray that we could inhale, that would cover the lungs and the airways with an antibody and that would block the virus in a way that everyone could do at home. You would not have to go to a hospital or a clinic or a doctor's office to do that. It would be something that you could do at home, just like you could do your asthma medicines or your inhaler at home.

Barr: What made you conceive of your study and when did you all first begin laying the groundwork for your experiment?

Brody: TJ, why don't you jump on that one.

Esparza: Gabrielle, as you know towards the end of March the NIH entered a maintenance phase as the number of COVID cases in the area were rising and our lab, of course, was part of that. We had a period where most of the scientists at the NIH were at home. We started having journal clubs and discussing our normal research, but on the side, we were also discussing if we could have thought about this in advance, were there any things from our own research that we could leverage to help join the fight against COVID-19? And Dave and I, through a variety of phone calls and texting—at first it was kind of a tongue-in-cheek, oh, what could we do—and then we realized, we do have a legitimate potential to apply our technology. So that was the early phase. That was the end of March, early April. Then what we did was we wrote a brief proposal and we submitted it to our Scientific Director, Lorna Role, who then encouraged us to write a more thought-out proposal, which was then submitted to the NIH COVID Committee. Then based on our application of what we were proposing to do, they approved us to come back to campus with a limited capacity. One of the key aspects that allowed us to do this was we would only have one individual in the lab doing this to protect staff, and so we were able to come in and get this work going. We started in mid- to late-April and worked through the summer.

Barr: That's really great. I had another question. What were the benefits of using antibodies derived from the camel family versus using antibodies produced through yeast?

Brody: I'm sorry, say that again.

Barr: What were the benefits of using antibodies that you all derived through the camel family versus using nanobodies that can be produced through yeast?

Esparza: I think you're referring to the production of the antibodies in yeast. Depending on the original source of an antibody, which typically will come from the camel family, which as Dave mentioned includes llamas, alpacas, vicunas, and dromedaries. Once you have those, it's the molecular biology that we apply in order to modify them as we grow them in a microorganism such as E. coli or yeast so that the antibody itself doesn't originate from yeast. It does originate from the llama. So, there is just a difference in understanding.

Brody: Just to also clarify a little bit, Cormac, the llama, is also really useful because we can immunize the llama and the llama has a terrific immune system where it can make lots and lots of thousands and millions of different antibodies. Then we can test those and figure out which ones are the best in the laboratory. But the llama makes a relatively small amount of each antibody whereas once we have the instructions, the blueprint for the antibody that we like, we can tell the yeast what to do and the yeast will make as much as we want.

Barr: Okay, does it make a difference what type of animal you use; a llama versus a camel versus an alpaca? Or it doesn't really make that much of a difference?

Brody: I don't know the answer to that. TJ, do you know?

Esparza: Certainly, any of the camels can produce nanobodies. Traditionally, alpacas and llamas have been used because of their moderate size relative to a camel. You could use a camel, but because of their body size it takes more material to immunize them. In the United States, at least, using camels as a service for making nanobodies is not existent to our knowledge. There is an investigator in the National Cancer Institute named Mitchell Ho. He does have a library that he has produced from camel that he has used for some of his experiments, but in terms of the services that are available commercially for immunizing animals, the llama and alpacas are the most abundant. It really just comes down to convenience.

Barr: Did you all have certain criteria when choosing the llama Cormac or camel in terms of what you were looking for?

Brody: It was based purely on looks. No just kidding.

Barr: He's very cute. I saw a picture.

Brody: I don't think we really choose the llama himself. Our collaborator Triple J Farms in the state of Washington had Cormac available. He had done good work in the past and that was it. It was their choice. We trust them to make the best choice. But no, we did not interview them or do camera tests.

Barr: I did not know if they had certain specifications that you were looking for, or if they had had certain illnesses in the past you would not have selected them or something like that.

Brody: I mean no, he was a young healthy llama, who has done good work in the past.

Esparza: Right, and similar to the NIH, they have their own animal studies committee, and they have their own vets that come. So, the animals go through the same rigors of care that we deliver to our animals here. As Dave mentioned, just ensuring that he had normal health parameters and he had been successfully used in previous projects.

Barr: Can you explain the strategy that your team developed to produce llama nanobodies that prevent SARS CoV-2 infections and how your methods were sort of like other researchers that are also using llama nanobodies?

Brody: Yes, go ahead TJ.

Esparza: Okay. Most of the researchers that have produced nanobodies, typically, what they will do is they'll choose the antigen or the protein of interest. In this case if you look at the structure for SARS-CoV-2, you hear a lot of people talk about the spike protein, which looks like the crown structure, that is where the corona and the coronavirus name comes from. The very tips of those spike proteins, that is what engages with the cells to cause infection. What we did was we used the spike protein to immunize our llama. In terms of what would differentiate our project from others is we used what we term a biased selection method, where in the test tubes, we were selecting for the nanobodies, we actually competed for interaction with the ACE-2 (angiotensin converting enzyme) receptor, which is what the coronavirus binds to, so that we could select four nanobodies that would block that interaction, and not select for nanobodies which would bind to other aspects or other parts of the spike protein. That would help increase or enrich the nanobodies for that blocking interaction. So really that would be the unique aspect in terms of what we have seen published so far.

Barr: Can you talk a little bit about the process of immunizing Cormac and testing the results and explain why you chose five times over a course of 28 days? Was the timing and the number of times that he was immunized significant?

Brody: Would you take that one again TJ?

Esparza: Okay. Whenever you immunize an animal with a protein, generally what we would do is we would take blood from the animal at intervals and test the blood to see if the animal is having an immune reaction or what is called an immune titer. In this case, part of our choice of immunizing weekly over a 28-day period was we really wanted to try to fast-track the project. Sometimes to get better nanobodies you might immunize over a longer period, more time between the intervals, months in some cases, people will go up to a year. Really it came down to a gamble. We wanted to accelerate the project, but we did have what we call test bleeds, which are small three to five ml [milliliter] blood samples that were sent to us. We were able to see a very strong immune response by week four. We had pretty good confidence that we were within the range that would give us a good response.

Barr: That's really great. So, of the 13 candidates that Cormac produced, you found that NIH-CoVnB-112 was the most effective at preventing COVID-19 infection. What inherent properties about this nanobody makes it so effective?

Brody: Mostly it is the one that binds the tightest. It just holds on the tightest. That was the most, the biggest criteria that we were looking for. But the second most important criteria, I think, is that we could produce a lot of it. It was a good expressor. I mean sometimes you make a protein, and it works pretty well, but it's just really hard to make enough of it to be useful. But this is a good one, because it binds really tightly, and we have not had any trouble making lots of it. It's been pretty stable to make it. Those are the two main criteria, but other than that, luckily, we have a good number of choices. Some of our other antibodies are also very good. It's always good to have a good Plan B and a Plan C.

Barr: Definitely. Can you talk a little bit about the testing aspect of your research when you tested it in the test tubes, in the petri dishes? What does that process look like?

Brody: Sure. TJ do you want to talk about our collaboration with Dr. Negin Martin down at NIEHS. This has been a terrific collaboration.

Esparza: There's a very brief back story. During the shutdown period when projects were being approved for COVID exemption, the investigators involved added their information to a dashboard, which other people who are part of that COVID research group were able to see. So, Dave and I were monitoring who was doing what and we found a great collaborator, Negin Martin, down at Triangle Park in North Carolina. She had developed a viral assay which uses a safe virus that expresses the spike protein from SARS-CoV-2, which could be used in an assay to measure whether or not you could block the binding or the infection of that virus. That allowed us to send material down and Negin was able to use her assay in a fluorescence measurement, basically measuring how much red fluorescence would be in a cell, which indicated an infection, and we were able to add different amounts of our lead antibody and see that we were able to block very efficiently the infection, and it blocked efficiently whether or not that was material that was in liquid form or whether it was material that had been aerosolized and then collected and then used to test. It gave us pretty good confidence that we could aerosolize our nanobody and potentially use it in the future for small animal studies and hopefully translate this down the road into humans.

Barr: That would be exciting. What challenges have you all experienced to date with this COVID project?

Brody: I mean there are always challenges for every puzzle.

This is, of all the things that I have worked on in my scientific career, this is actually one of the things that went the smoothest. It seems like most things just worked right away. I mean part of that is because TJ is really good at this kind of thing, and part of it is because Cormac, the llama, did a terrific job. Part of it is that the whole world's attention is focused on this question. So, when we called people up, companies that needed to provide reagents or potential collaborators, usually picked up the phone right away and were happy to help. Right away we were able to move a lot faster in this project than on some of the other projects.

I think the biggest challenges are still to come to be honest. The biggest challenges in my view are going to be what happens when this product gets into humans. It is very hard to predict whether a new product is going to be safe in humans or not. We think it is going to be safe, but it's really hard to predict. The big challenge, I think, will be is it safe to use in humans? And there is no way to tell until we get there. Lots of things go pretty far along the line and then they don't work in the end because they cause some side effect or some toxicity or they cause an immune response; somebody's allergic to it. I think our biggest challenges are still to come.

Barr: Were you all surprised by some of your initial results, being so strongly positive?

Brody: Yes, we were surprised because we have struggled a lot to make nanobodies to other proteins and it has been really hard work, and in this case like bang, right off the bat, we had terrific nanobodies. We were surprised, yes.

Esparza: It was the middle of June when we had our first indication that we had real binders. From the end of April through May we did the immunization and then we did all the steps required to isolate the nanobodies. Then we had some preliminary results. I remember it was a pretty warm June evening and Dave and I were on a phone call. We were looking at data together and we were both like, “This looks great, but we have to do this several more times because we just didn't believe that it worked that well the first time.”

Brody: That's right. My famous quote, you probably can put it on my gravestone is, “That's great, now do it again.” I just could not believe it was that good. It's like, oh, come on, TJ do it again. I can't be right.

Barr: That's really great. You spoke a little bit about how some of your laboratory findings can be translated to practical treatments like using inhalers, but you said that it could also maybe be used for testing. Can you talk a little bit more about that?

Brody: I mean sure, one of the really great things about nanobodies is that you can make them really cheaply and they are really stable. At high temperatures they still work. At low temperatures, they still work. You can leave them out at room temperature, they still work. You can dry them down, they still work. They are really very robust. So we think they potentially could work very well in test kits like those point-of-care test kits that people use because they're so stable and so cheap. We think they could be used for environmental sensors, sensors out in the air to detect how much COVID-19 there is floating around in the air. In fact, we have got a collaborator, a funded project with one of our collaborators at Washington University, St. Louis, to try to develop those kinds of environmental sensors. We think they could be useful for coating surfaces; that would put a protective coating on some surfaces. If you sprayed it on the surface, that would protect the surface. Yes, we think there is a lot of possibilities.

Barr: That's really exciting. Can you speak a little bit about why you selected nanobodies from the IgG (Immunoglobulin G) versus the IgA (Immunoglobulin A) class that is common in protecting the respiratory tract?

Brody: The nanobodies—I think they are really only one flavor. I mean there is really only just the heavy chain, only nanobodies. That's so in camels, llamas; these are circulating nanobodies that circulate around in the bloodstream. They are not usually in their native state. I don't think they are especially involved in the respiratory tract. We have got the idea though for a while for how to do this from another group that was making nanobodies to treat another virus called respiratory syncytial virus or RSV, which is a common cause of severe lung infection in babies. This other group had been making nanobodies and inhaling them in a sheep model of RSV and also had gone into human trials for inhaled treatment of RSV. That was our inspiration. Well look, they work in an inhaled form even if that's not how the llamas were using them in nature.

Barr: Okay, that's interesting. You spoke a little bit about some of the uses potentially from your research. What are some of your next steps?

Brody: The big step that is going on right now is testing these nanobodies in hamsters. Hamsters have attracted a lot of attention because the coronavirus can infect hamsters. It doesn't really affect mice and rats particularly well, but it affects hamsters and monkeys pretty intensely, and a few other types of animals as well. Right now, as we speak, there are some tests going on in hamsters by our collaborators and we have more tests and hamsters coming up. The way those tests are going, or and will go, is that the hamsters will get a dose of the nanobody, inhale a dose of the nanobody, and then about 24 hours later, they will get exposed to the real coronavirus in a biosafety laboratory where it's safe to work on deadly viruses. Then they will get another dose of the nanobody and we'll see; some of the hamsters will get the protective nanobodies and some will not. We will see if they do any better if they get the protective antibodies. We will also see if they have any toxicity. This will be a chance to see whether that is toxic in the hamsters as well. That is the key thing right now is does it actually work with a real coronavirus in an actual animal.

Barr: Is this being done on site or with a collaborator somewhere else in the United States?

Brody: Yes, with collaborators elsewhere in the United States. Here we don't have the facilities to do the biosafety testing; for example, we don't have the facilities to work with live deadly virus. That's too dangerous for us to do in the laboratory. That has to be done by collaborators elsewhere.

Barr: Okay, I guess we will transition from your science to your role as scientist. What were your roles in this project respectively?

Brody: TJ did everything and I cheered him on. Do you want to elaborate more on that, TJ?

Esparza: Sure, Dave and I have been working together for almost 14 years and we have a pretty close working relationship. Whenever we approach these projects, we can kind of anticipate the appropriate next steps and we communicate on a very regular basis. This was something that we were really able to do, even though there was only one person in the lab where I was really the hands. There was constant communication back and forth with Dave. Dave was networking with other experts in the field and constantly pulling in advice and information. We were able to run experiments carefully, look at the results, adjust what we were doing, and then calibrate the next set of experiments very, very quickly. This was not a typical situation where you might have a postdoc in the lab and it's more of an educational environment. This was more of a we-were-sprinting. We were trying to move things as quickly and efficiently as we could given the limitations of only having one person. Now of course we mentioned Negin Martin as a collaborator; we were able to pull other people in to help do some of the work alongside to confirm things and also do independent assays for us. Dave may minimize his role, but it was very important for that relationship to work well.

Brody: Our collaborators at Naval Medical Research Labs have also played an important role. I mean we are relatively new in the world of nanobody research. They have been doing this sort of thing for a long time. It was really important. We shipped some stuff over to them and they tested it in their hands. Again, it was really important that we see it does not just work in our hands. It works in their hands too.

As I said we never believe anything until we see it more than once, in more than one person's hands. It was really great to have the collaborators over at Naval Research Labs and also our collaborators in North Carolina showing the same thing. There is a lot of networking involved, but again, like I said, because it's kind of a crisis situation, all hands-on deck to use a Navy term. People were really willing to put it at the top of their list and then get it done.

Barr: That's really great. Personally, what have been some highlights and lowlights for you all during the pandemic?

Brody: TJ, you want to jump on that one?

Esparza: I guess to clarify the question—do you want the answer in reference to the work specifically?

Barr: It can be either—not really about your project, but if you feel professionally what would have been some highlights or lowlights or even just as a person living through the pandemic. We all have had challenges and opportunities.

Esparza: I guess in terms of highlight—I'm really sorry to say that this would be a highlight, it is kind of a double-edged sword because of the situation. I wish that we were not in this situation. But you know we had an extraordinary opportunity as neuroscientists to engage in something that really impacts potentially the global community. There were periods of time when I was the only person in the lab. Building 10 was very quiet. There was almost a reverence to the work in that we knew that we were in a privileged position to be able to do this. We are of course always privileged to pursue science. That was a highlight.

We were able to stay engaged and we were able to feel that we could have an impact, whereas, I know there is a lot of anxiety in the scientific community for colleagues, who felt like they were on the sidelines, who weren't able to engage. Dave and I communicated this too at some level. But you know one of the things that [if] really anytime something wouldn't work, there was a personal level of, I do not want to say guilt, but we wanted to push this as quickly as we could, because I think daily, we were seeing the number of people in the U.S. and around the world succumbing to COVID. I think of having that constantly sitting on your shoulder. Certainly, we're not driving the only thing out there, but I think it really causes you to reflect. Most of the time in science, we don't see a direct impact. It takes time whereas this was happening and is happening in real time. That is kind of my view.

Brody: Yes, and I agree. The highlights have been, “Hey, this is all hands-on deck, let's jump in.” This is a once in a lifetime opportunity. It reminded me of the times during, for example, World War II, two of my famous favorite scientists, Alan Hodgkin and Andrew Huxley, were electrophysiologists. They worked on radar during World War II because it was the same kind of thing; it was all hands on deck, everybody's got to get in there and win this. Then they went back to working on squid, ion channels, and electrophysiology after the War was over. We had that kind of same highlight feeling like wow, here we are all hands-on deck. Let's do this.

Some of the low points have been we are just not moving fast enough. We are moving as fast as we can, but it's so frustrating when things don't move as fast as we would like them to and that's still frustrating. Now I personally think we could move a lot faster than we are. We are constantly pushing to try to move even faster.

Another really unfortunate lowlight, emphasizing the point that things are not moving as fast as we would like, is one of my good friends just lost both of his parents at a nursing home They were in a nursing facility and one of the nursing facility workers tested negative and had no symptoms and came to work and spread the virus to 40 or 50 people, about half of whom died in the nursing facility, including both of his parents. That really hit me personally. This is one of my good friends. So, it can't come soon enough.

Barr: How quickly do you think some of the concrete results of your product will be on the market like those inhalers or things to coat the surfaces or things like that? It seems like it will take some time.

Brody: It will. I think that a lot is going to depend on whether we can find the right commercial partner. One of the things that is terrific about the NIH is we have amazing resources, and amazing people, and opportunity to have a lot of scientific freedom. But one of the disadvantages about the NIH is we do not have a lot of ability to do commercialization directly. We have to find partners to commercialize this sort of thing. I think if we find the right commercial partner, I think it could go pretty fast. If we don't find the right commercial partners, it will never get onto the market. That is the big question.

Barr: Definitely. One of my last questions is has there been anything that you have learned from your COVID research that you envision can be applied to tackling other diseases?

Brody: TJ thought about that right away, so I'll let you give the first try on that one. I can tell you what he told me. You know what TJ told me real early on is that this is great, and this approach might really work well for SARS-CoV-3, which is a virus that doesn't exist yet, but probably will come in the future at some point. The idea is how we could be better prepared in the future. Imagine a scenario in which people scour the world for new viruses, and even if we don't know if they are going to turn into pandemics or not, we start making antibodies, nanobodies for them, test them and stockpile them and so if they do turn into pandemics, we're already ready. We were proactive as opposed to being reactive.

Yes, we have been very reactive to this virus. We have not been proactive in any way. If we had known in the time of the original SARS in 2003, if we had known that there was going to be a SARS-CoV-2, we could have been much more proactive back then starting in 2003 to start doing this kind of thing, but we didn't know. You know as my old friend Todd Rasmussen says, if you want an apple, the best time to have planted an apple tree would have been 15 years ago, but the second-best time is today. I think we could be very proactive in the future for preventing other diseases like this and using these nanobodies for a whole host of other applications.

Barr: Of course. Is there anything else that either of you would like to share as scientists but also as people, who are living through this pandemic like other Americans?

Esparza: I think one of the important things that we have learned in this period is that as scientists we tend to live in silos where we have a combination of doing independent work and working with closely related investigators. But what we have seen a lot in the last year is that people from a variety of fields have come together to work as efficiently and over time that efficiency has improved. One of the things that has really inspired me is that we have learned that if and when there is a big problem that needs to be dealt with—we can think of big problems like cancer, Alzheimer's disease—as a group of scientists, we can integrate as a network very efficiently and focus a lot of energy on solving a problem. I think there has been a lot of technologies, a lot of ideas, a lot of out-of-the-box thinking that has really been highlighted in the last year. I think that should act as a seed for future innovation.

That's something that I think is a positive that will come out of this.

Brody: And I agree. I think one of the things we have learned is how much we can do via the internet, via Skype, and Zoom, and phone calls and things like that; just how effective we can be in networking and science in our scientific communications. This is very new. I mean if this had happened 10 years ago or even five years ago, it would have been a lot harder, so I think what we are seeing is just how powerful our communications technology is now to the actual practice of science. That has been eye-opening for me and I think we will view this time as a pivot point where we never go back to doing it the way it was before, even when we can, because we have just seen how powerful these new communication technologies are.

Barr: Thank you both for your time and thank you both for your service. I wish you the best success with your research and I hope that you and your families continue to stay safe.

Brody: Thank you and likewise to you. I appreciate your time and your efforts to communicate this important work to the public.